Expanded materials and methods:

Animals: The *erk5-flox* mice were previously described (ERK5flox/flox)[1]. ERK5flox/flox mice on a C57bl/6 background were mated with mice expressing Cre under cardiac α-myosin heavy chain (α-MHC) promoter to generate cardiac-specific ERK5 knockout mice (ERK5-CKO)[2]. The α-MHC-Cre mice on C57bl/6 background were kindly provided by Dr. E. Dale Abel[3]. Rat wild type and dominant negative mutant (K94A/K447A) p90RSK1 cDNA was subcloned into a pBluescript-based Tg vector between the 5.5-kb murine-α-MHC promoter and 250-bp SV-40 polyadenylation sequences (a kind gift from Dr. J. Robbins, Children's Hospital Research Foundation, Cincinnati, Ohio) as we have previously described[4]. The mouse constitutively active form of MEK5α (CA-MEK5α, S311D/T315D) cDNA was subcloned into a pBluescript-based Tg vector between the 5.5-kb murine-αMHC promoter and 250-bp SV-40 polyadenylation sequences, and we generated three different lines of CA-MEK5α-Tg in FVB strains. All three lines showed a similar phenotype and MEK5α expression as we reported previously[5]. The animal studies were performed in accordance with the University Committee on Animal Resources and institutional guidelines at University of Rochester Medical Center.

Antibodies: Antibodies were purchased from the following vendors: anti-p90RSK1 (C-21, #SC-231), anti-Ub (P4D1, # SC-8017), anti-HA (Y-11, SC-805), anti-CHIP (H-231, SC-66830), and anti-VP16 (#SC-7546) from Santa Cruz (Santa Cruz Biotechnology, CA); anti-phospho-p90RSK (Ser380, # 9341), anti-phospho-ERK5 (Thr218/Tyr220, #3371L), anti-ERK5 (#3372), anti-cleaved caspase 3 (# 9664), and anti-Bcl-2 (#2876) from Cell Signaling (Cell Signaling Technology Inc, Danvers, MA); anti-tubulin (T-5168) and anti-Flag (# F-3165) from Sigma (Sigma, St. Louis, MO); anti-ICER antibody was generated as previously described[6].

Reagents: Angiotensin II was purchased from Calbiochem (Cat # 05-23-0101, an Affiliate of Merck KGaA Darmstadt, Germany). D-glucose (Dextrose) and Mannitol was from Sigma (St. Louis, MO). Hydrogen Peroxide was purchased from Sigma (#216763). GST fused active recombinant full-length rat RSK1 (Cat # 14-479, Lot # 31236AU) was purchased from Millipore (Millipore, Billerica, MA).

Plasmid and adenovirus vector construction: Mouse ERK5 (mERK5a) and a constitutively active form of MEK5α (CA-MEK5α) were cloned as previously described[7]. Both the VP16 full-length wild type and truncated forms of mERK5a in the pACT vectors were generated by inserting the mouse full-length wild type and truncated forms of mERK5a isolated from pcDNA3.1-mERK5a into BamHI and NotI sites of the pACT vector. Glutathione S-transferase (GST)-ICER was created by ligation of fragment from BamHI-EcoRI digest of pCMVtag2b-ICER into the same sites in the pGEX-KG vector (Amersham Biosciences, GE Healthcare Biosciences, Pittsburgh, PA). ERK5 S496A point mutations were created from the mERK5a full-length using the QuikChange site-directed mutagenesis kit (Stratagene). For adenovirus preparation, DN-p90RSK construct was cloned into an AdEasy-CMV system (QBIOGen, Carlsbad, CA) with Sal*I* and Hind*III* restriction enzymes[8]. All constructs were verified by DNA sequencing. Adenovirus vector containing β-galactosidase (Ad-LacZ) was used as a control virus.

Cell culture: Primary cultures of neonatal rat ventricular cardiomyocytes were performed as described previously[9, 10]. Briefly, neonatal rat ventricular cardiomyocytes were obtained by enzymatic digestion of cardiac ventricles from 2 -3 day old Sprague-Dawley rat neonates. The ventricular tissues were subjected to multiple rounds of enzymatic digestion using collagenase II (1.2 mg/ml; Worthington Biochemicals Corp., Lakewood, NJ, Cat # 4177). Cardiomyocytes were collected after every two rounds of collagense digestion by centrifugation at 800 rpm for 5 min at 4 ºC. Non-myocytes were removed by pre-plating on culture dishes. Cardiomyocytes were then plated on pre-coating gelatin plates, in DMEM low glucose supplemented with 10% fetal bovine serum (FBS) and 10 μM cytosine 1-β-D-arabinofuranoside (Sigma). Addition of cytosine 1-β-D-arabinofuranoside can inhibit the growth of contaminating non-myocytes.

More than 90% of cells were cardiomyocytes (positive for α-actinin). Next days, cardiomyocytes were washed thoroughly with PBS to remove any unbound cells, then maintained for another 3 days to let the cells stable before using for experiments. HeLa cells were maintained in DMEM (GIBCO, Carlsbad, CA) supplemented with 10% FBS, 50 units/mL penicillin, and 50 μg/mL streptomycin.

ICER mRNA detection: Rat neonatal cardiomyocytes cultured on 6-well-plates pre-coated with gelatin at ~ 80% density. After 24 hours, cells were washed thoroughly with PBS. Three days later, cells were infected with either Ad-LacZ as a control (MOI=20) or Ad-DN-p90RSK (MOI=20) overnight, followed by stimulation with Ang II (200 nM) for 30 min. Total RNA was then isolated using RNeasy Plus Mini Kit (Cat. # 74134, QIAGEN Sciences, Maryland 20874, US) according to the manufacturer's instruction. Genomic DNA was eliminated by gDNA Eliminator column, which is included in the Kit. Single strand cDNA synthesis was done in 50 μL reaction volume containing 2 μg of purified RNA, 5 μL 10X buffer, 11 μL $MgCl_2$, 10 μL dNTPs, 2.5 μL random hexamer, 1.25 μL oligo dT, 1 μL RNAse inhibitor, and 0.75 μL reverse transcriptase enzyme (TaqMan Reverse Transcription Reagents, N808-0234, made for Applied Biosystems by Roche Molecular systems, Inc., Branchburg, New Jersey, US). Target cDNA levels were quantified by real-time RT-PCR using a MyiQ^{TM}2 Optics Module (Bio-Rad Laboratories, Inc.). Each reaction mixture (20 μL) contained cDNA synthesized from 20 ng of total RNA, 10 μL of iQ^{TM}SYBR Green Supermix (Bio-Rad Laboratories, Inc.), 0.5 μmol/L of each primer. The real-time PCR protocol consisted of an initical step at 95^{0}C for 3 min, followed by 40 cycles: 95^{0}C for 10 sec and annealing at 55^{0}C for 30 sec. The ΔΔCt method was used to calculate the fold change in mRNA expression: ΔCt = Ct (target gene) – Ct (housekeeping gene), ΔΔCt = ΔCt (treatment) - ΔCt (control), fold change = $2^{(-_{\Delta\Delta}Ct)}$. Primer sequences are as follows: rat ICER forward: 5'-ATG GCT GTA ACT GGA GAT GAA ACT G -3' and reverse: 5'-CAC CTT GTG GCA AAG CAG TA-3'; Rat β-Tubulin forward: 5'-GGA GGA TGC TGC CAA TAA CT-3' and reverse: 5'-GGT GGT GAG GAT GGA ATT GT-3' [11].

Mammalian two-hybrid analysis: The association between ERK5 and CHIP was analyzed using a CheckMate mammalian two-hybrid system (Promega Corporation). A pBIND vector containing Gal4-DBD was fused with the full-length of CHIP. pACT vector containing VP16 was fused with either full-length or truncated forms of ERK5. Sub-confluence HeLa cells plated in 12-well-plates were transfected using Opti-MEM (Invitrogen, Carlsbad, CA) containing Plus-Lipofectamine mixture with PG5-Luc vector and various pBIND and pACT plasmids in the presence or absence of pHA-CA-MEK5α, as indicated, for 4 hrs. Cells were then washed and fresh DMEM medium supplemented with 10% FBS was added. The pG5-Luc vector contains five Gal4 binding sites upstream of a minima TATA box, which in turn, is upstream of the firefly luciferase gene. Since pBIND also contains the *Renilla* luciferase gene, the expression and transfection efficiencies were normalized to the Renilla luciferase activity.

The association between ERK5 and p90RSK was analyzed using the same method as for analysis ERK5-CHIP association, using pBIND vector contains Gal4-DBD was fused with the full-length p90RSK. Cells were collected 24 hours after transfection unless otherwise stated, and the luciferase activity was assayed with the dual luciferase reporter system (Promega, Madison, WI, USA) using a TD-20/20 Luminometer (Turner Designs, Sunnyvale, CA, USA). Transfections were performed in triplicate, and each experiment was repeated at least thrice.

Immunoprecipitation and Western blot analysis: Cells were washed 3 times with phosphate-buffered saline (PBS), and cell extracts were prepared in modified radioimmunoprecipitation assay 1 (RIPA) buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1 % Nonidet P-40, 0.1% sodium dodecyl sulfate (SDS), 1:200-diluted protease inhibitor cocktail (Sigma, MO, USA), 1 mM PMSF and 10 mM NEM). Mouse hearts were washed 3 times with cold Hanks' Balanced Salt Solution (HBSS) (Mediatech. Inc, Manassas, VA 20109). After the hearts was frozen in liquid nitrogen, the hearts were ground with mortar and pestle into powder. This heart powder was homogenized in 1 mL of modified RIPA buffer,

followed by sonication (5 x 10 second pulse) and then centrifuged at 14,000g (4^0C for 30 min), and protein concentration was determined as previously described[12]. Immunoprecipitation with anti-ERK5 antibody or anti-CHIP antibody was performed as described previously[10, 13]. Bound proteins were released in 2x SDS sample buffer, resolved by SDS-polyacrylamide gel electrophoresis, transferred onto a Hybond enhanced chemiluminescence nitrocellulose membrane, and visualized by using the Enhanced Chemiluminescence Substrate (PerkinElmer, Shelton, CT, USA) according to the manufacturer's instructions. Immunoblotting was performed as previously described[14].

p90RSK *in vitro* kinase assay: p90RSK activity was measured by autophosphorylation and GST-ERK5 phosphorylation as described previously [15]. GST-ERK5 wild-type and mutants (1 μg) were resuspended in 50 μL of kinase assay solution containing 10 mM/L $MgCl_2$, 10 mM/L $MnCl_2$, and 25 mM/L HEPES (pH 7.4), and the kinase reaction was initiated by adding 1 μg active RSK1 (Rsk1/MAPKAP Kinase 1a, active, Cat # 14-479, Millipore), 15 μM/L ATP (Cat # 10519979001, Roche Applied Science, Indianapolis, IN), and 3 μCi [γ-^{32}P]ATP. The reaction at 30°C was for 30 min and terminated by adding Laemmli's sample buffer. Auto-phosphorylation and GST-ERK5 phosphorylation were determined by densitometry at the correct molecular weights in the linear range of film exposure with the use of a scanner and NIH Image J.

***In vitro* ubiquitination assay with GST-ICER:** Lysates of neonatal rat ventricular myocytes or ground frozen heart tissues were prepared in modified radioimmunoprecipitation assay (RIPA) butter [50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 1% Nonidet P-40; 0.1% sodium dodecyl sulfate (SDS); 1:200-diluted protease inhibitor cocktail (Sigma, St. Louis, MO); 1 mM PMSF; and 10 mM NEM]. Lysates were then immunoprecipitated using an anti-CHIP antibody and incubated overnight at 4^0C with gentle shaking. After that, 30 μL of the 50% protein A and G agarose mixture (1:1 volume ratio) (protein A agarose - Cat # 15918-014, Invitrogen; protein G agarose - Cat # 15920-010, Invitrogen) was added and incubated for another 2 hrs, with gentle shaking. The immunoprecipitated CHIP was then washed five times using washing buffer [50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM EDTA; 0.1% Nonidet P-40; 0.1% sodium dodecyl sulfate (SDS)]. GST-ICER protein (500 ng) was added at the last washing step. After that, the washed beads were subjected to *in vitro* ubiquitination assay to determine CHIP Ub E3 ligase activity using the Ubiquitin-Protein Conjugation kit (Boston Biochem, Cambridge, MA, USA), following the manufacturer's protocol. Briefly, the combined immunoprecipitated CHIP and GST-ICER were incubated with a ubiquitin and E1/E2 enzyme mixture in an energy buffer for 90 min at 37°C, with gentle shaking. The reaction was then stopped by adding 2X SDS loading buffer followed by Western blot analysis with an anti-ubiquitin antibody. Since bands over 300kDa were close to the stacking gel and were often not clearly resolved, we excluded bands over 300kDa from quantification. As shown in Fig.2, we found the dimer of GST-ICER around 70 kDa, but non-specific IgG bands dominated the area around 40-60kDa. Therefore, we selected the area from 70-280 kDa for quantification.

Permanent coronary ligation surgery: Myocardial ischemia was induced in the mouse by permanent ligation the left coronary artery: The mouse was placed onto a heating pad (half inch plexiglass between the animal and the heating pad). Oral intubation was established using a PE 90 tube (o.d. 1.27 mm) advanced slowly into the trachea. Mechanical P.I. ventilation (tidal volume of approximately 250μl at 130 breaths/min) was begun. Maintenance anesthesia was 1.5% isoflurane. After intubation, a left thoracotomy was performed in the fourth intercostal space. The mouse heart was exposed, and the left coronary artery was ligated intramurally 2 mm from its ostial origin with 9-0 silk suture. The suture was secured to occlude the coronary artery. After confirming that the LV color change and ECG S-T segment elevation, the chest was closed with 6-0 coated vicryl suture, the skin was closed using 6-0 nylon, the anesthesia was stopped and the mouse was allowed to recover for several minutes before the endotracheal tube was removed. A sham operation involved an identical procedure, except that the suture was passed through the myocardium without occlusion. Animals were monitored by ECG during surgery. Aseptic

technique was used throughout. Animals were placed on a warming pad for all procedures to reduce the risk of hypothermia.

Streptozotocin (STZ) injections: Hyperglycemic mice (8-12 weeks) were made by a single dose intraperitoneal injection (IP) of freshly prepared STZ solution (150 – 200 mg/kg body weight in citrate saline, pH 4.5) using 26.5 Gauge needle. Diabetic status was confirmed by measuring blood glucose at day 7 of STZ injection.

Analysis of apoptosis: Cardiomyocyte apoptosis was measured by the terminal deoxyribonucleotide transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) detecting in situ DNA fragmentation. TUNEL staining was performed using the In Situ Cell Death Detection Kit, Fluorescein (Cat # 11 684 795 910; Roche Diagnostics, IN, USA) as described previously[16]. For the counter staining of cardiomyocytes, cells were also stained with anti-cardiomyocyte-specific sarcomeric anti-actinin antibody. Briefly, cardiomyocytes or the coronal sections of frozen hearts were washed three times with cold PBS and fixed with a freshly prepared paraformaldehyde (4%) in PBS for 30 min, followed by permeabilization with 0.1% Triton X-100 in PBS for 5 min. Cardiomyocytes or heart sections were then incubated with blocking buffer (5 % goat serum in PBS) for 60 min to block nonspecific binding. Incubation with the primary antibody (anti-actinin antibody, 1:5000 dilution in 1% goat serum/PBS) was performed at 4°C overnight. After that, Cardiomyocytes or heart sections were washed three times with PBS. Then, secondary antibody labeled with Alexa Fluor 546 dye against mouse IgG_1 (Molecular Probes; 1:2000 dilution) was added and incubated for 60 min at room temperature. After washing, cells were applied to TUNEL staining following the manufacturer's instruction. Cells were mounted using mounting medium for fluorescence with DAPI (Vectashield, H-1200, Vector Laboratories, Inc. Burlingame, CA 94010). All of the images were taken on an Olympus BX51 upright fluorescence microscope equipped with an 40x objective lens. Quantification of the overlay image was done using ImageJ software (National Institute of Health). An average of 500 anti-a-actinin antibody positive cells from random fields were analyzed. All measurements were performed blinded and at least three independent experiments were performed.

Echocardiographic analysis: Echocardiographic analysis using M-mode was performed using a Vevo 2100 echocardiography machine from VisualSonics (Toronto Canada) equipped with a MS-550D probe. Echocardiography (M-mode image) of mouse left ventricle was obtained in un-anesthetized mice. LV function was measured in the parasternal short axis view at midlevel. %FS was assessed by measurement of the end-diastolic and end-systolic diameter (end diastolic diameter (Dd) – end systolic diameter (Ds))/ end-diastolic diameter x100%). %EF was assessed by Dd^3-Ds^3/Dd^3. We collected and averaged the data from 5 heart beats per trace, and three traces from each animal. The pooled data were analyzed for statistical significance.

Assesment of infarct size: The harvested hearts were fixed with 10% formalin for one day, dehydrated in ascending ethanol series, and embedded in paraffin. The tissues were sliced at 5 μm thickness from apex to basement into three transverse slices. The Sections were stained by Masson's trichrome staining for a determination of infracted region. Slides were analyzed and photographed using an Olympus light microscope (Model BX41). The length of entire circumference and infarcted area were quantified using ImageJ software. The MI size was evaluated from each of three slides[16].

LC-MS/MS analysis of ERK5 phosphorylation by p90RSK: Kinase reactions were performed in 1.5 mL LoBind tubes (Eppendorf, Hauppauge, NY). GST-ERK5 fusion protein (1 μg) was attached to glutathione-agarose resin. The beads (30 μL packed volume) were washed with 50 mM ammonium bicarbonate to remove any protease inhibitors left after protein purification. The beads were then resuspended in 100 μL of the kinase assay solution, and the kinase reaction was initiated by the addition

of 1 μg active RSK1and 400 μM/L ATP. After 30 min of kinase reaction at 30°C, the reaction buffer was removed from the beads, which were then washed with 50 mM ammonium bicarbonate three times. For LC-MS/MS analysis, the GST-ERK5 fusion protein on beads was resuspended in 20 μL of solution containing 50 mM ammonium bicarbonate and 188 ng trypsin and was digested overnight at 37°C. The sample was reduced by 2 mM DTT for 30 min, with shaking at 60°C, alkylated by 10 mM iodoacetamide (IAA) for 30 min in the dark at room temperature (RT), and finally quenched by 10 mM cysteine for 30 min at RT in the dark. A second aliquot of 188 ng of trypsin was then added and digested for further 3 hr. Chymotrypsin digestion was performed in the identical manner. Digested samples were lyophilized and resuspended in 10 μL of 5% acetonitrile and 0.05% formic acid, and 15% of this peptide digest (1.5 μL) was loaded on a Magic C18 AQ (Michrom) nanospray tip, packed to 5 cm. This tip was loaded, using a pressure bomb and washed after installation on the HPLC with 5% methanol and 0.1% formic acid for 10 min with a flow rate of 600 nL/min (about 10 column volumes = 6.6 μL). The peptides were eluted and analyzed by an LC-MS/MS run, using a 5-15 % methanol gradient over 2.5 min, followed by a 15-60% methanol gradient for 67 min, a 60% methanol isocratic step of 4 min, and ending with a 3 min 95% methanol step, with all solvents containing 0.1% formic acid. A full MS survey scan was performed every 3 seconds and the top 7 peaks were selected to produce an MS/MS fragmentation spectrum. The top three fragmentation spectra that experienced a neutral loss of 98 daltons were further fragmented to produce an MS/MS/MS spectrum.

The MS and fragmentation spectrum data was used in a Mascot search of the human proteome to identify peptide sequences modified by phosphate groups. The following search criteria were used for selecting fragmentation spectra that mapped to phosphorylated peptides: peptide tolerance = -0.8 to +0.5, a minimum ion score of 15, and a fragmentation spectrum containing fragment ions that either included or flanked the phosphorylated amino acid position. Representative fragmentation spectra are included in Figs.4B and 4C. Phosphorylation sites that mapped to the same ERK5 protein position in both the trypsin and chymotrypsin-treated samples were regarded as confirmed phosphorylation sites.

Statistical Analysis: Data are reported as mean ± S.D. Statistical analysis was performed with the GraphPad Prism program, version 4.00 (GraphPad Software, Inc. CA). Differences were analyzed with 1-way or 2-way analysis of variance (ANOVA) with repeated measures, followed by Tukey's multiple comparison test. P values less than 0.05 are accepted as being statistically significant and indicated by one asterisk (*). Those less than 0.01 are indicated by two asterisks (**).

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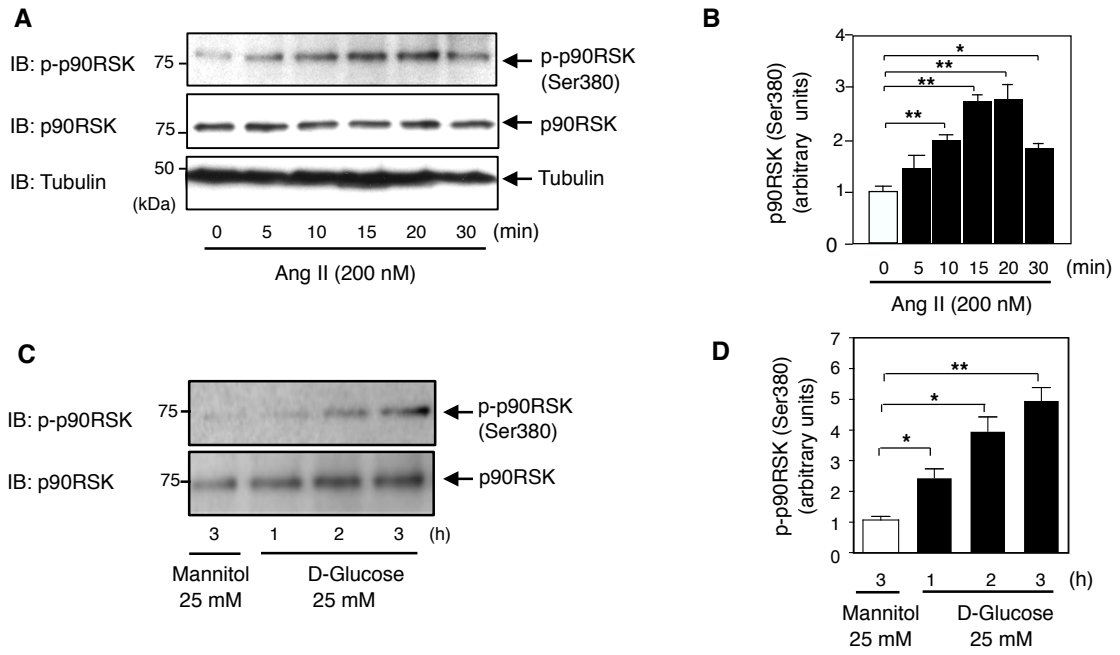


Fig. I. p90RSK activation in cardiomyocytes (**A-D**) Cardiomyocytes were stimulated with Ang II (200 nM) **(A-B)** or high glucose or mannitol **(C-D)** for the indicated times. p-p90RSK, p90RSK and tubulin were detected by Western blotting of total cell lysates with each specific antibody. **(B, D)** Intensities of p-p90RSK protein bands were quantified using a Fujifilm image-analysis program (Image Gauge 4.0) and were calculated relatively to the intensity of the tubulin band at each time point. Results were expressed as fold increase of p-p90RSK in AngII-treated cells compared to untreated cells. Shown is mean ± S.D. (n = 3). ** p <0.01, * p <0.05 compared to untreated cells.

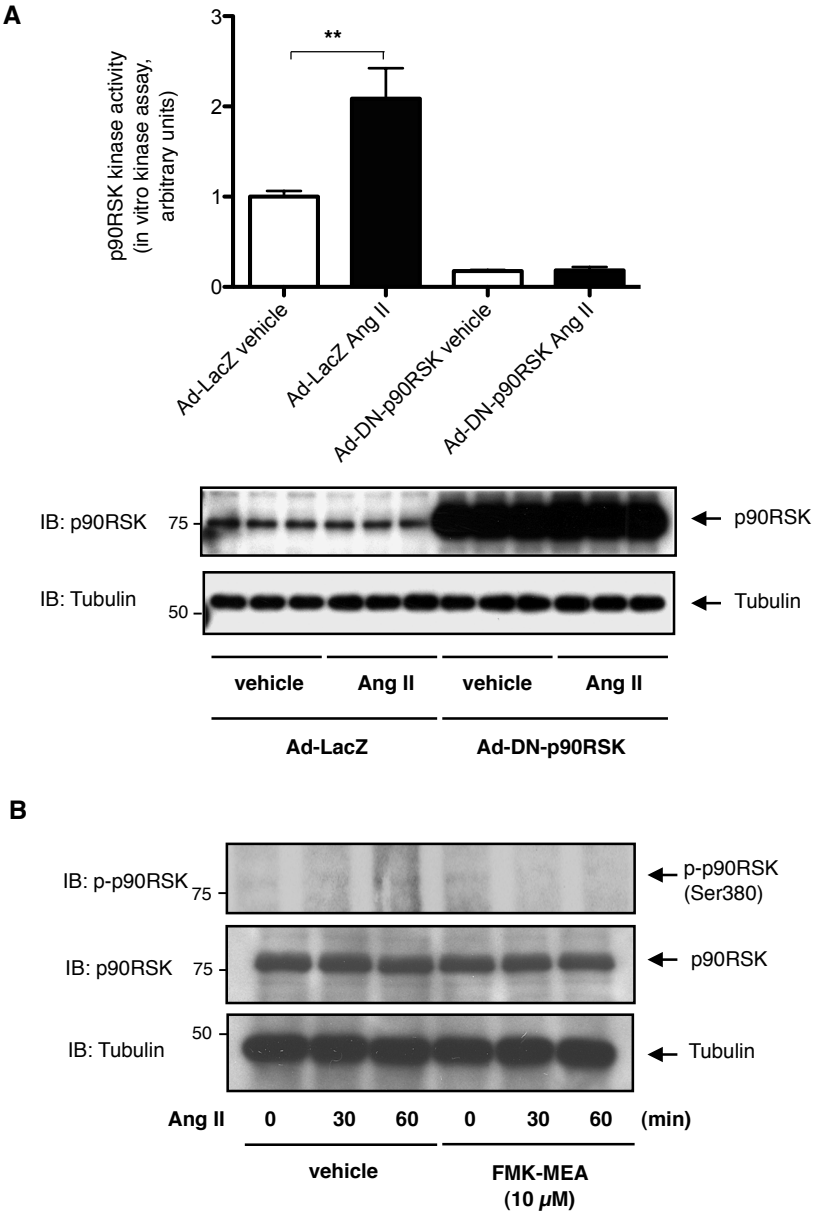


Fig. II. Ad-DN-p90RSK and FMK-MEA, a specific p90RSK inhibitor, blocked Ang II-mediated p90RSK activation in cardiomyocytes (**A**) Cardiomyocytes were stimulated with Ang II (200 nM), and p90RSK kinase activity was detected using an in vitro kinase assay as described in methods. The p90RSK kinase activity (upper) was normalized to its expression level, which was evaluated by Western blotting of p90RSK (lower). **(B)** Cardiomyocytes were pretreated with vehicle or FMK-MEA for 3 hrs and stimulated with Ang II (200 nM) for the indicated times. p-p90RSK, p90RSK and tubulin were detected by Western blotting of total cell lysates with each specific antibody. The blots are representative of data obtained from three separate experiments.

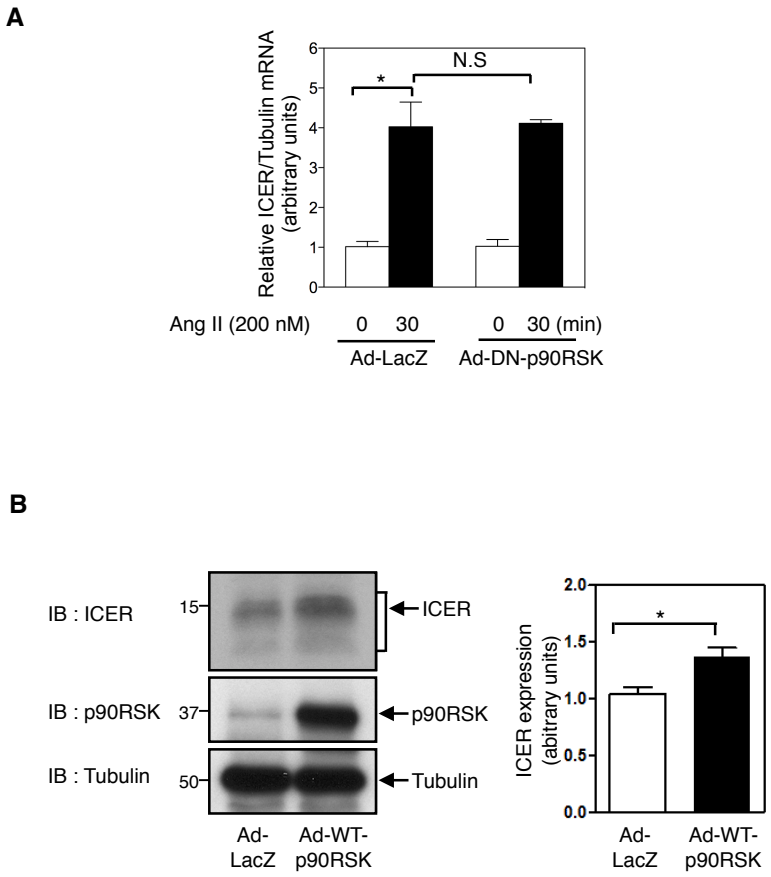


Fig. III. Ad-DN-p90RSK did not inhibit Ang II-mediated ICER mRNA expression in cardiomyocytes (A), but Ad-WT-p90RSK enhanced ICER protein levels (B). (A) Cardiomyocytes were transduced with Ad-LacZ or Ad-DN-p90RSK for 24 hrs. Cells were then stimulated with Ang II (200 nM) for 30 min, and ICER mRNA level was detected by qRT-PCR as described in methods. (**B**) Cardiomyocytes were transduced with Ad-LacZ or Ad-WT-p90RSK for 24hrs. ICER, p90RSK and tubulin were detected by Western blotting using the total cell lysates with each specific antibody (left). Intensities of ICER protein bands were quantified using a Fujifilm image-analysis program (Image Gauge 4.0) and were calculated relatively to the intensity of the tubulin band at each time point. Results were expressed as fold increase of ICER in Ad-WT-p90RSK-transduced cells compared to the Ad-LacZ-transduced cells. Shown is mean ± S.D. (n = 3). * p <0.05.

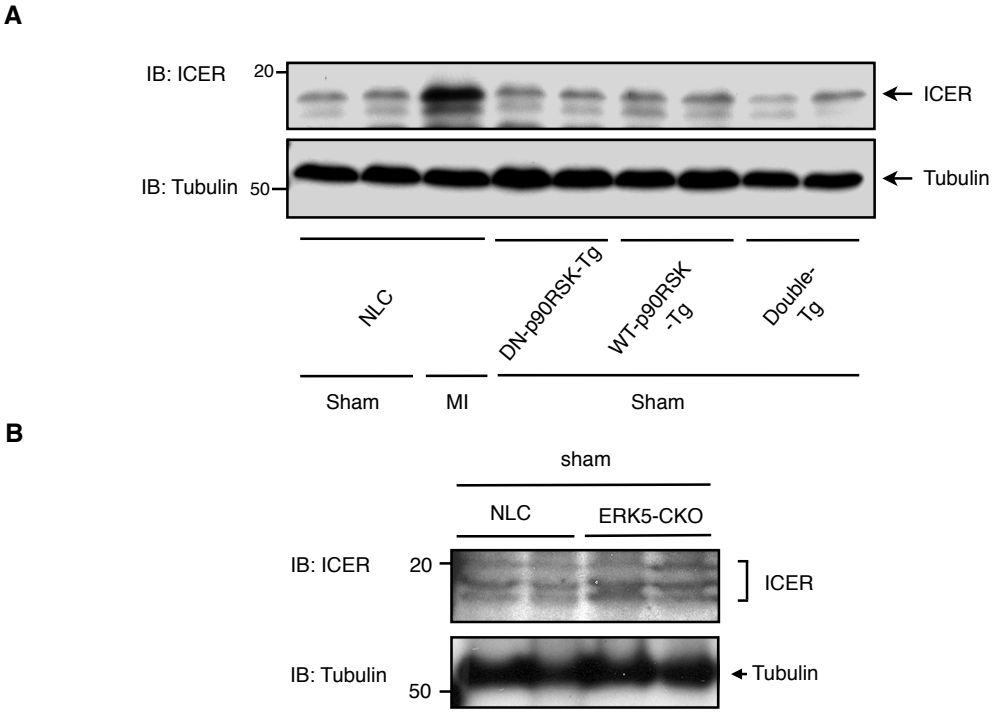


Fig. IV. DN-p90RSK-Tg, WT-p90RSK-Tg, Double-Tg, and ERK5-CKO mice showed no difference of ICER expression in sham operated mice. Expression of ICER and tubulin in heart samples collected from sham and MI in NLC, sham in DN-p90RSK-Tg, WT-p90RSK-Tg and Double-Tg mice **(A)**, and heart samples from sham in NLC and ERK5-CKO **(B).**

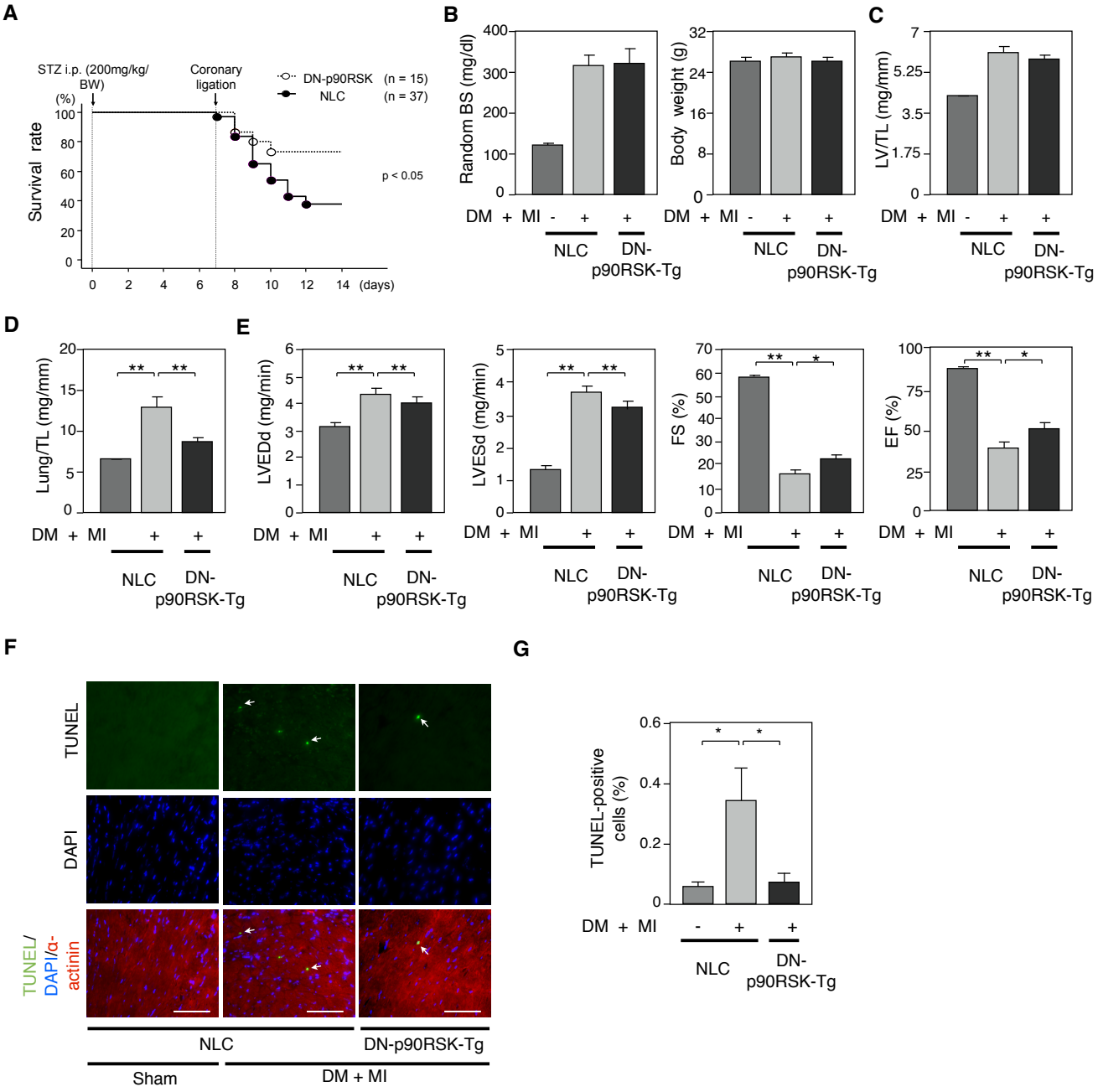


Fig. V. Inhibition of p90RSK activation prevented the exacerbation of LV dysfunction after MI in diabetic mice. (**A**) Kaplan-Meier survival analysis in diabetic NLC (n=37) and DN-p90RSK-Tg (n=15) after MI. Overall survival was significantly higher in DN-p90RSK-Tg compared to NLC mice. * p <0.05 compared to NLC group. (**B**) Random blood sugar (BS) and body weight at one week after STZ injection for coronary ligation (DM + MI) or vehicle-treated sham operation groups in NLC or DN-p90RSK-Tg mice. (mean ± S.D., n =9-10) (**C**) LV weight/TL (mean ± S.D., n =9-10) and (**D**) lung weight/TL in DM + MI or vehicle-treated sham operation groups in NLC or DN-p90RSK-Tg mice one week after surgery. (**p <0.01, mean ± S.D., n=9-10). (**E**) Echocardiographic data obtained from DM + MI, or vehicle treated sham operation groups in NLC or DN-p90RSK-Tg mice one week after surgery. LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; TL, Tibial length. (*p<0.05, **p <0.01, mean ± S.D., n=9-10).(**F**) Cardiomyocytes apoptosis in the remote area was increased in DM + MI group, which was inhibited in DN-p90RSK-Tg mice. TUNEL-positive cardiomyocytes were counted in the remote area as described in Fig.6D. Representative pictures of TUNEL (top), DAPI (middle), and merged of α-actinin with TUNEL and DAPI staining (bottom) of the remote area from NLC mice subjected to sham or DM+MI, and DN-p90RSK-Tg mice subjected to DM + MI operation. 40X objective lens. Scale bars: 40 μm (**G**) A bar graph showing TUNEL-positive cells (%) in NLC and DN-p90RSK-Tg. (* p <0.05, mean ± S.D., n =3).

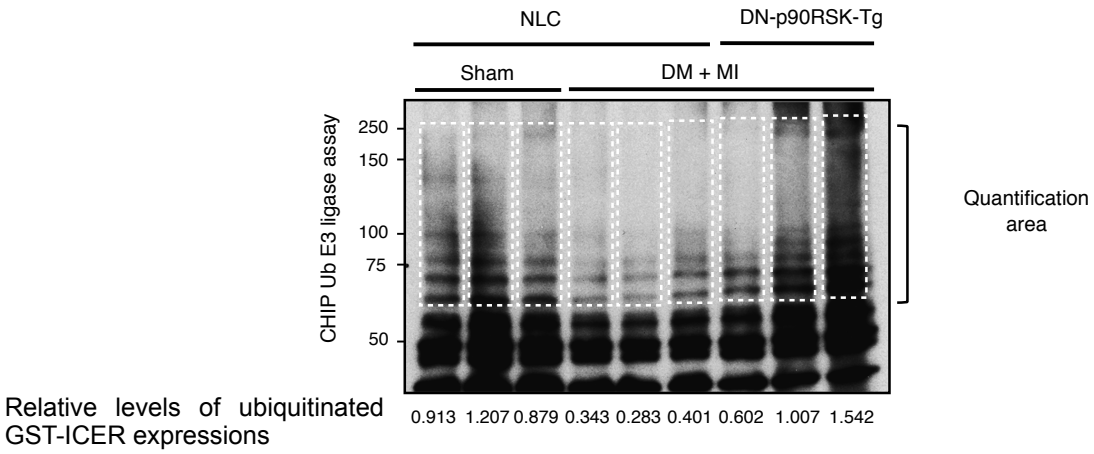


Fig. VI. Quantification of CHIP ubiquitin E3 ligase activity. The activities of CHIP Ub E3 ligase were assessed by quantifying ubiquitinated GST-ICER fusion proteins. Briefly, protein was extracted by modififed RIPA buffer, and then immunoprecipitated with anti-CHIP antibody. CHIP proteins were immunopresipitated by protein A and G agarose mixture. After that, GST-ICER protein as added to each sample. CHIP Ub E3 ligase activity was detected using Ubiquitin-Protein Conjugation kit. Science Lab 2001 Image gauge software (version 4; Fuji Photo Film, Tokyo, Japan) was used for the analysis. Ubiquitinated GST-ICER expressions were calculated in equal area described in white broken squires. Relative levels were indicated as based on means of NLC sham mice.

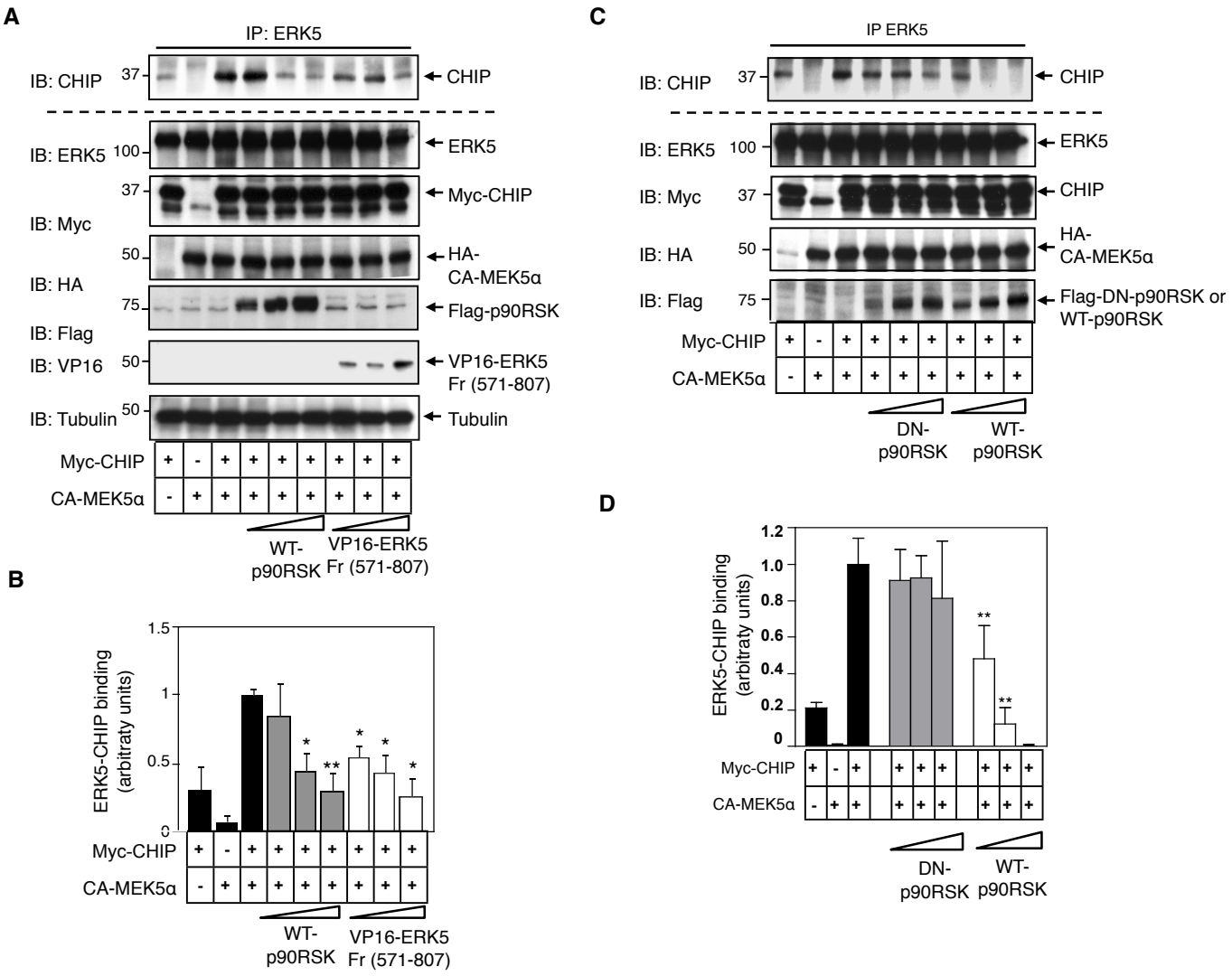


Fig. VII. p90RSK kinase activation inhibits ERK5-CHIP association. (**A**) Both wild type p90RSK (WT-p90RSK) and ERK5-Fr (aa571-807) disrupted ERK5-CHIP association. After plasmids were transfected as indicated, cell lysates will be immunoprecipitated with anti-ERK5 and then immunoblotted with anti-CHIP. Protein expression was determined by immunoblotting with each specific antibody. **(B)** Relative ERK5-CHIP binding was quantified as described in Fig.2D (mean±S.D., n=3, (**p<0.01, * p<0.05 compared to both Myc-CHIP and CA-MEK5α transfected cells (the third black bar from left). Results are expressed as fold decrease in ERK5-CHIP binding in WT-p90RSK (grey bars) and ERK5-Fr (aa581-807) (white bars) transfected cells compared to the control cells (the third black bar from left). (**C**) WT-p90RSK but not DN-p90RSK fully disrupted ERK5-CHIP interaction. ERK5-CHIP binding and each protein expression were detected as described in (A). (**D**) Relative ERK5-CHIP binding was quantified as described in Fig.2D. Results are expressed as fold decrease in ERK5-CHIP binding in cells over-expressing WT-p90RSK. **p<0.01, mean±S.D., n=3.

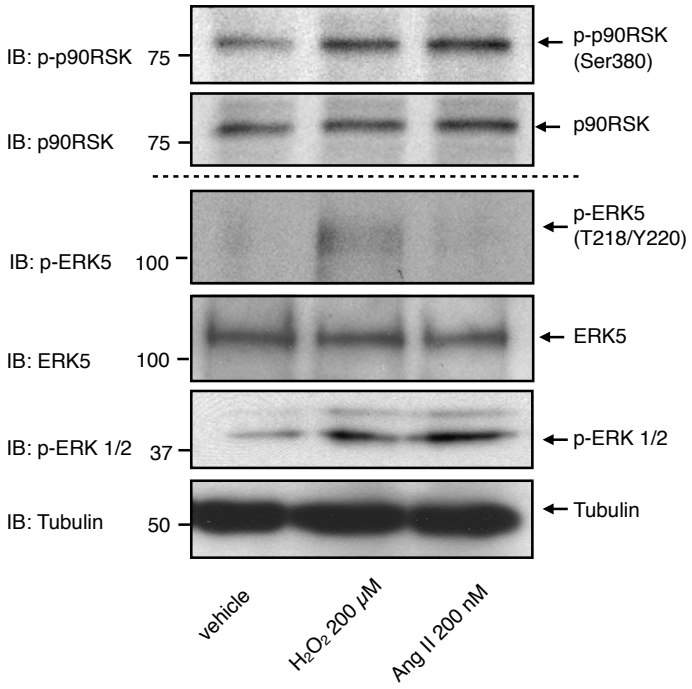
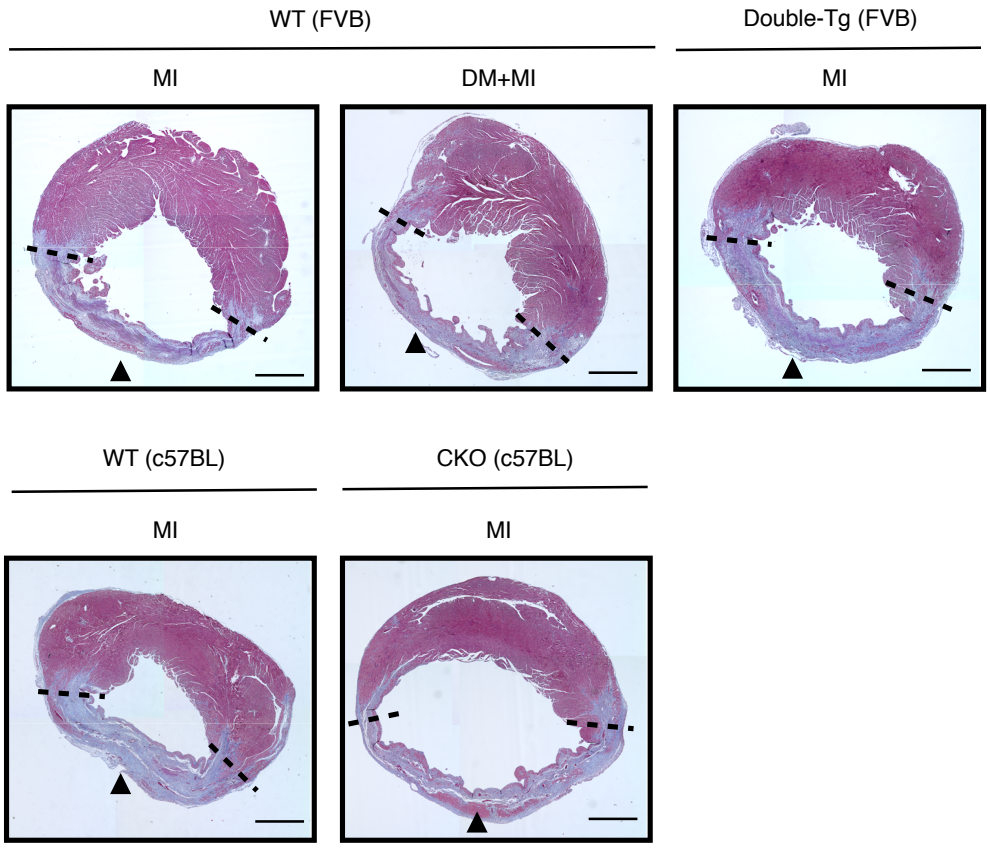


Fig. VIII. Ang II increased only p90RSK but not ERK5 kinase activity in cardiomyocyte. Cardiomyocytes were stimulated with Ang II (200 nM) or H_2O_2 (200 μM) for 15 min. p-p90RSK, p90RSK, p-ERK5, ERK5, p-ERK1/2, and tubulin were detected by Western blotting using the total cell lysates with each specific antibody. Representative immunoblots from duplicate experiments are shown.

A



B

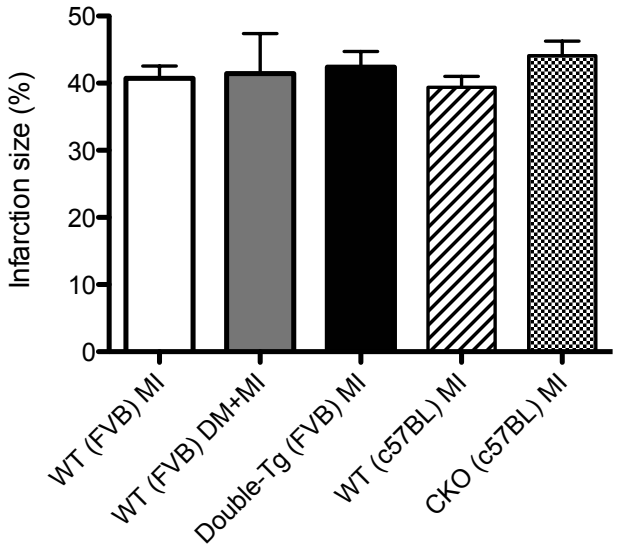


Fig. IX. Evaluation of infarction size at one week after MI surgery.
(**A**) Representative pictures of LV tissue sections by Masson's trichrome staining. Infarct areas were described in black triangle between broken lines. 4X objective lens. Scale bars: 1000 µm. (**B**) Comparision of MI sizes. The sizes were not significantly changed in each groups. Data are shown as means ± S.D. (n=3).